POLYMORPHIC TRYPANOSOMES OF THE *T. BRUCEI* GROUP RECOVERED FROM THE MWANZA SLEEPING SICKNESS AREA

BY

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INTRODUCTION

The trypanosome strains dealt with in this paper were recovered from the Sleeping Sickness area in the neighbourhood of Mwanza in Tanganyika Territory.

During a tour through the fly-belt in July and August, 1922, I isolated four different strains, all of which were inoculated into experimental monkeys. The strains included two from man, one from wild fly, and one from wild game. The monkey carrying the wild game strain escaped; the other strains reached the Laboratory at Entebbe, and form the subject of the studies here set forth.

Several papers have been published dealing with the Mwanza outbreak of the human trypanosomiasis (Swynnerton, 1923 a and b; Duke, 1923a). The disease in man was generally acute and fatal, and, in places, attained epidemic proportions. The causative trypanosome, when inoculated into guinea-pigs, showed posterior-nuclear forms, and was accordingly diagnosed as of the nature of *T. rhodesiense,* rather than *T. gambiense.* The outbreak presented many points of interest. The obscurity surrounding the origin of the human parasite; its apparently sudden appearance, and its mode of propagation; the occurrence of a *T. rhodesiense* like organism in epidemic form, and so far north; the discovery that the insect vector was a new species, *G. swynnertoni*—all these features combined to produce a novel and fascinating problem.

The first part of the present paper deals with the three strains during their sojourn at the Entebbe Laboratory. They have been studied in relation to both the mammalian and the insect host. Incidentally, in the course of these studies, further information has been obtained about the behaviour of trypanosome strains when subjected to direct transmission over a prolonged period.

Part II represents an attempt to assemble and review the data hitherto obtained by the experimental study of directly-transmitted strains, and to set forth the conclusions resulting from these studies.

PART I

A.—ACCOUNT OF THE MAINTENANCE OF THE STRAINS AND THEIR ANIMAL REACTIONS

(1) Human Strain, 483.

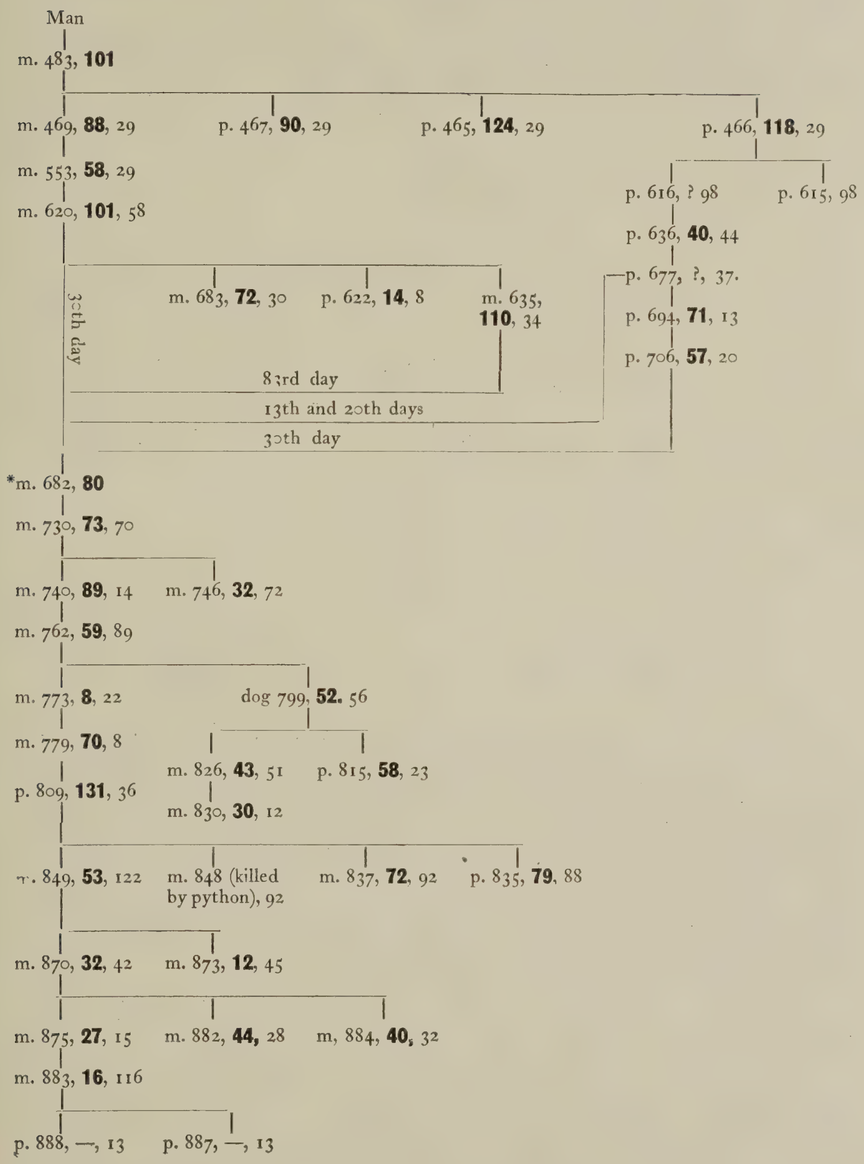
Inoculated on 1.8.22 from young adult native S. of Nyamagana village in the fly area.

The upkeep of this strain is shown in Table I.

The figures in black type give the duration of the disease in the animal; the other figure gives the day of the disease in the infecting animal when the sub-inoculation was made.

TABLE I.

Human Strain 483.



* Monkey 682 received in all 5 inoculations, as shown in the Table.

NOTE. In this and other similar Tables m. signifies monkey, and p. guinea-pig.

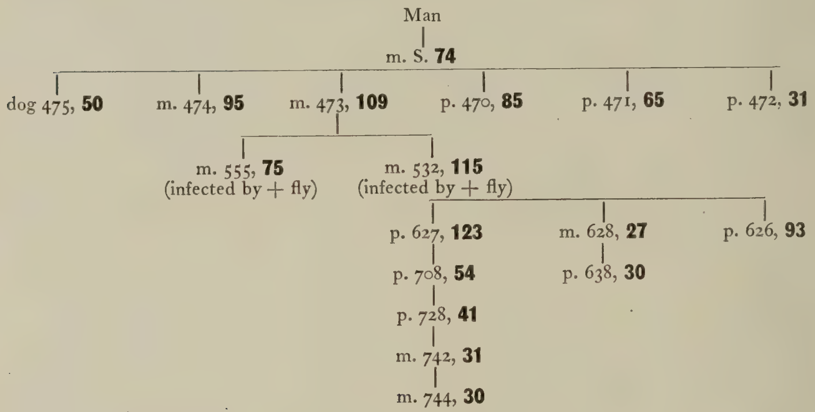
(2) HUMAN STRAIN S.

Monkey S. was inoculated on 2.8.22 from an adult male native M., of Gamboshi village in the fly-belt.

Table II shows the upkeep of this strain until July, 1923, when circumstances necessitated its abandonment.

TABLE II.

Human Strain S.



AVERAGE :—

8 completed monkeys	69.5 days.
8 completed guinea-pigs	65.2 days.
1 dog	50 days.

A young chimpanzee was fed upon by infective flies in Experiments 527 and 530 (of Tables IV and V), on two consecutive days. Trypanosomes were seen in its blood six days later, and it died sixty days after infection. This chimpanzee seemed quite well up to the day when its companion died (*cf.* below); from then on it began to mope and refuse its food. It is difficult to decide to what extent grief influenced the course of the infection.

Both the natives from whom these two human strains were derived denied knowledge that they were sick; they were detected at the inspection musters held at villages in the fly-belt. They both had enlarged cervical and axillary glands, and looked as though they had recently lost flesh. The disease was, to all appearances, not very advanced. The Senior Commissioner, Mwanza, informs me that native S. died on 12.2.23, and native M. during December, 1922.

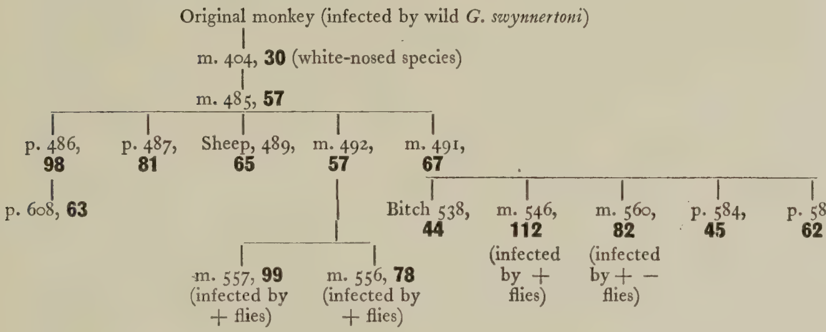
(3) WILD FLY STRAIN, E.

Recovered by feeding wild *G. swynnertoni* on a clean experimental monkey. Trypanosomes appeared first in the monkey's blood on 26.7.22.

Table III shows the maintenance of this strain, which was relinquished in January, 1923.

TABLE III.

Wild Fly Strain E.


Original monkey (infected by wild *G. swynnertoni*)
m. 404, **30** (white-nosed species)
m. 485, **57**
p. 486, **98**
p. 487, **81**
Sheep, 489, **65**
m. 492, **57**
m. 491, **67**
p. 608, **63**
Bitch 538, **44**
m. 546, **112** (infected by + flies)
m. 560, **82** (infected by + − flies)
p. 584, **45**
p. 585, **62**
m. 557, **99** (infected by + flies)
m. 556, **78** (infected by + flies)

AVERAGE :—

7 monkeys	78.8 days.
5 guinea-pigs	69.8 days.
1 bitch	44 days.
1 sheep (native)	65 days.

An old chimpanzee, fed upon by flies (Experiment 531, Tables VIII and IX), on a single occasion, became infected and died forty-eight days later. Four infective flies fed on this animal, and trypanosomes were already numerous in the peripheral blood on the ninth day after infection.

It is plain from Tables I, II and III that the three strains, when first isolated, were essentially identical in their behaviour in laboratory animals. Nor is any difference discernible in the morphology of the strains.

(4) POSTERIOR-NUCLEAR FORMS :—

All these strains have been examined at intervals for posterior-nuclear forms, two hundred trypanosomes being inspected in each of a series of stained thin blood-films from infected guinea-pigs. In all the strains these forms were found, though latterly not at every examination. In some guinea-pigs they were less common than in others. In guinea-pig 809 of Table I they were generally difficult to find. In the case of strain 483, posterior-nuclear forms are apparently less common now than formerly ; for example, they were always common in guinea-pig 835 of Table I, but rarer and often of less pronounced type in guinea-pigs 887 and 888.

Thus the Mwanza human strain 483 can still produce posterior-nuclear forms, though it has lost the power of cyclical development in tsetse ; it is, however, not quite clear whether the two processes are entirely independent. Now it is interesting to note that both the Direct Transmission Strain and the Antelope Strain ceased to produce posterior-nuclear forms in guinea-pigs in the later stages of the upkeep of these strains at the laboratory. It has been suggested elsewhere (Duke, 1921) that these two strains are descendants of trypanosomes which, at the time of the great epidemic in Uganda, were capable of infecting man, and that they have assumed certain of the characteristics of *T. brucei* as the result of long-continued and intimate association with *Tragelaphus spekei.* On the other hand, the Mwanza human trypanosomes are indistinguishable by laboratory tests from *T. brucei.* It is tempting to see, in the observations just recorded, confirmation of these views on the origin of the Uganda and Tanganyika strains, respectively. The power exhibited by the two former of producing posterior-nuclear forms, being a comparatively recent acquisition, is soon lost ; whereas, in the case of the Mwanza strain, the characters of typical *T. brucei* are more deeply rooted, and so persist longer under radical changes of environment.

These studies also show that increase of the virulence of a strain of trypanosomes is not necessarily associated with the formation by the strain of posterior-nuclear forms in suitable animals.

B.—ACCOUNT OF TRANSMISSION EXPERIMENTS CARRIED OUT WITH LABORATORY-BRED *G. PALPALIS*

(1) Human Strain 483.

Table IV sets forth transmission experiments carried out with this strain shortly after its arrival at the laboratory, and Table V gives the results obtained by dissecting the positive flies obtained in these experiments.

Table IV.

Experiment No.	Infecting monkey	Date	Flies dissected			Day on which dissection began	Alive 25th day	Result of feeding on clean monkey	Date at which box became infective	Flies containing flagellates	Duration of experiment
			Males	Females	Total						
503	468	11–13.9.22	22	23	45	12	42	+	26	1	38
527	469	21–23.9.22	26	34	60	8	54	+	18	9	36
530	468	23–25.9.22	35	32	67	7	59	+		7	34
539	469	26–28.9.22	37	31	68	11	66	+	20	3	32
*583	469	11–12.11.22	23	35	58	16	54	—	—	2	66
*586	469	12–13.11.22	26	31	57	16	53	—	—	6	66
*588	469	13–14.11.22	35	31	66	23	63	—	—	3	64

The brackets indicate that the two boxes were fed upon the same clean monkey on alternate days.

Note.—The * indicates that the flies in these three boxes were fed throughout the experiment upon a baboon, an animal not susceptible to this strain of trypanosomes.

TABLE V.

Experiment No.	No. of fly containing flagellates	Day of experiment on which fly was dissected	Intensity of infection		Sex of fly
			Gut	Glands	
503	1	38	+++	+++	Male
527	2	27	+++	+	Female
—	3	32	+++	+++	Male
—	4	32	+++	+++	—
—	5	33	+++	++	—
—	6	36	+++	+++	—
—	7	36	+++	+++	Female
—	8	36	+++	+++	—
—	9	36	+++	+++	—
—	10	36	+++	—	—
530	11	7	++	—	—
—	12	7	++	—	—
—	13	34	+++	+++	Male
—	14	34	+++	+++	—
—	15	34	+++	+++	—
—	16	34	+++	+++	Female
—	17	34	+++	+++	—
539	18	32	+++	+++	Male
—	19	32	+++	+++	Female
—	20	32	+++	+++	—
583	21	15	+++	—	Male
—	22	40	+++	—	—
586	23	41	+	—	—
—	24	48	+	—	—
—	25	48	+	—	—
—	26	66	+++	+++	—
—	27	66	+++	+++	Female
—	28	66	++	—	—
588	29	39	+++	++	Male
—	30	44	+++	+++	—
—	31	62	+++	+++	—

(2) HUMAN STRAIN S.

Table VI gives the transmission experiments with this strain, and Table VII the results of dissecting the positive flies obtained.

TABLE VI.

Experi- nent No.	Infecting monkey	Date	Flies dissected			Day on which dissection began	Alive 25th day	Result of feeding on clean monkey	Date at which box became infective	Flies containing flagellates	Duration of experi-ment
			Males	Females	Total						
521	473	19–21.9.23	31	28	59	10	46	+	25	2	32
528	473	22–24.9.23	30	31	61	10	44	+	19	7	29

TABLE VII.

Experiment No.	No. of flies containing flagellates	Day of experiment on which fly was dissected	Intensity of infection		Sex of fly
			Gut	Glands	
527	1	32	+++	+++	Male
—	2	32	+++	+++	—
528	3	20	+++	+++	Female
—	4	28	+++	+++	Male
—	5	28	+++	+++	—
—	6	28	+++	+++	—
—	7	28	+++	—	Female
—	8	29	+++	+++	Male
—	9	29	+++	+++	—

(3) WILD FLY STRAIN E.

Table VIII gives the transmission experiments, Table IX the results of the fly dissections with this strain.

TABLE VIII.

Experiment No.	Infecting monkey	Date	Flies dissected			Day on which dissection began	Alive 25th day	Date at which box became infective	Flies containing flagellates	Result of feeding on clean monkey	Duration of experiment
			Males	Females	Total						
513	491	16, 17, 18.9.22	21	19	40	12	34	—	0	—	44
{526	491	21–23.9.22	32	39	71	8	62}	22	{23	+	35
531	492	23–25.9.22	41	23	64	9	54		7		33
{540	491	26–28.9.22	40	18	58	25	56}	23	{3	+	34
542	492	27–29.9.22	30	43	73	8	55		5		34
{544	489	28, 29, 30.9 and 1 and 3.10.22	28	23	51	7	44}	22	{4	+	34
545	489	28.9–1.10.22	34	29	63	8	53		0		35
{547	492	30.9–2.10.22	22	26	48	8	37}	26	{1		32
548	491	2.10–4.10.22	32	35	67	6	52		3	+	29

The brackets indicate that the two boxes were fed upon the same clean monkey on alternate days.

TABLE IX.

Experiment No.	No. of fly containing flagellates	Day of experiment on which fly was dissected	Intensity of infection		Sex of fly
			Gut	Glands	
526	1	10	+++	(not seen)	Female
—	2	35	++	—	Male
531	3	14	+++	—	Female
—	4	18	+++	—	—
—	5	33	+++	—	Male
—	6	33	+++	+++	Female
—	7	33	+++	+++	Male
—	8	33	+++	+++	—
—	9	33	+++	+++	—
540	10	33	+++	+++	—
—	11	32	+++	+++	—
—	12	32	+++	+++	Female
542	13	17	+++	—	Male
—	14	20	+++	—	Female
—	15	34	+++	+++	—
—	16	34	+++	+++	—
—	17	34	+++	+++	—
544	18	20	—	—	Male
—	19	33	+++	—	—
—	20	34	+++	+++	—
—	21	34	—	—	—
547	22	20	+++	—	Female
548	23	26	+++	+	Male
—	24	29	+++	+	—
—	25	29	+++	+	Female

It will be seen from Tables IV-IX that all the three strains were readily transmissible by *G. palpalis* at the time of their isolation from the fly-belt.

The experiments arrayed in Table X were carried out in March and April, 1924, with the only strain surviving at that time, Human Strain 483.

TABLE X.

Experiment No.	Infecting monkey	Day on which dissection of flies began	No. of flies alive on 20th day	Flies dissected			Duration of experiment days	Day of disease in infecting monkey when flies put on
				Males	Females	Total		
1	837	14	45	27	22	49	31	36–38
2	848	29	50	24	26	50	31	7–9
3	837	9	47	24	27	51	30	39–41
4	848	11	55	29	30	59	32	10–12
5	849	22	62	33	29	62	32	11–13
6	837	9	65	42	30	72	33	42–44
7	848	8	50	25	29	54	33	13–15
8	849	9	68	35	34	69	36	14–16
9	837	8	43	44	12	56	37	45–47
10	849	11	54	30	29	59	36	17–19
11	848	10	51	38	23	61	39	17–19
12	837	6	56	36	27	63	38	49–51
13	848	8	57	34	33	67	38	20–22
14	849	9	41	33	26	59	38	21–23
15	837	8	32	25	27	52	42	52–54
16	848	11	57	34	27	61	41	23–25
17	837	11	47	36	23	59	35	55–57
18	848	9	56	38	29	67	35	26–28
19	849	8	47	26	33	59	37	28–31
20	837	11	56	34	26	60	38	58–60
21	837	9	50	36	19	55	36	61–63
22	849	9	30	24	22	46	37	31–33
23	849	10	58	37	30	67	38	32–34
24	837	10	60	29	34	63	38	63–65
25	849	12	31	15	19	34	37	35–37
26	837	9	19	12	16	28	38	65–67
	Totals	... ...	1287	800	682	1482		

Of the 1,482 flies dissected during these experiments, six were found to contain flagellates. In every case, however, the organisms were exceedingly scarce, amounting to a few individuals in the whole preparation of the teased-out gut. The particulars of these six flies are as follows :—

1. *Experiment* 2 : One female killed on thirty-first day after first infective feed.
2. *Experiment* 6 : One eleventh day male.
3. *Experiment* 8 : One ninth day male.

4 and 5. *Experiment* 9 : One tenth day male, and one thirty-fifth day male.

6. *Experiment* 10 : One twenty-second day male.

The minute infections shown by these flies were very remarkable. They were only revealed by a careful search of the freshly-teased gut, and sometimes only two or three flagellates were seen. They must be regarded as abortive attempts at development by a strain which has all but completely lost its power of maintaining itself in the alimentary canal of *Glossina*. When a normal fly-strain is exposed to tsetse the trypanosomes die out at once in the majority of the flies ; but, in the favourable minority, development proceeds uninterruptedly, until the gut of the insect is swarming with flagellates.

The condition in these six flies is quite different from anything hitherto observed, and represents a still further stage in the degeneration process already demonstrated in earlier work done with the Antelope Strain (Duke, 1923b).

PART II

A.—RESUMÉ OF EXPERIMENTAL WORK ON DIRECT TRANSMISSION OF TRYPANOSOMES

The experiments set forth in this paper complete a series of three parallel studies on the effect of continued direct transmission on polymorphic trypanosomes of the *brucei* group. The strains examined are the Direct Transmission Strain, isolated from wild Lake-shore *G. palpalis* in January, 1920; the Antelope Strain, isolated from a situtunga antelope on Damba Island in September, 1920; and the Mwanza Human Strain, 483 (Duke 1921, 1923a and 1924).

All these strains, when first isolated, were readily transmissible cyclically by laboratory-bred *G. palpalis* at the Entebbe Laboratory. A comparison of the histories of the three strains since their arrival at the Laboratory is interesting.

(1) Direct Transmission Strain.

After 11 direct passages, covering 16 months, was still cyclically transmissible by tsetse.

After 23 more passages, over a further period of 14 months, was no longer able to develop at all in tsetse. The exact period at which this change set in was not determined. A sudden increase of virulence appeared about the eighteenth passage, after 23 months' maintenance by direct transmission. This increase in virulence apparently occurred at about the time that the trypanosomes lost their power of developing in tsetse.

(2) Antelope Strain.

After 24 direct passages, over a period of about 22 months, had lost the power of invading the salivary glands of the fly; heavy gut-infections were still obtained, but here also there were signs of impairment in developing power. Ten months later, after 20 more passages, all power of development in tsetse had disappeared. The exact date at which this last stage was reached was not determined.

The virulence of this strain, in monkeys, apparently began to increase about the seventeenth direct passage, *i.e.* after about

15 months of maintenance. The average duration of the disease in the 24 monkeys employed during the first 16 direct passages was 34 days; during the subsequent 34 passages, extending over a period of 20 months, the average duration for 20 monkeys was 24 days.

It is impossible to determine with certainty if the virulence began to increase coincidently with the onset of impairment in the power of cyclical development in the fly. By the time the trypanosome had lost its hold on the salivary glands of the fly, virulence had begun to increase, and it may well be that the two processes were intimately associated.

(3) MWANZA HUMAN STRAIN.

Table V suggests that the strain had already undergone slight modification in relation to tsetse towards the end of its sojourn in monkey 469, *i.e.* during the second direct passage from man, and only two months after isolation. This inference is, however, by no means certain, as the baboon's blood may have influenced the development of the flagellates. The passage to the next monkey of the series was made by flies cyclically infected from 469. Eleven months later, after eight direct passages, the strain had all but completely lost the power of development in tsetse. The virulence of this strain in monkeys showed no increase at the time that the last transmission experiments (shown in Table X) were carried out. But in the passages immediately following, an increase was at length manifest, and the strain has been now transferred to guinea-pigs.

It will be noted that the Mwanza Strain lost the power of cyclical development in tsetse much more rapidly than did the two Uganda strains. Both the latter were undoubtedly cyclically transmitted previous to their isolation in monkeys. On the other hand, there are reasons for suspecting that Strain 483 may have been subjected to a number of direct passages from man to man in the fly-belt before reaching native S, and this may explain the relatively rapid alteration of this strain after its arrival at the laboratory.

B.—DISCUSSION

The results obtained from these transmission experiments, though of scientific interest, are not necessarily valid for human trypanosomiasis. Monkeys cannot be regarded as true natural hosts of the trypanosome ; and propagation by the syringe differs in many respects from any natural method of transmission. We are, indeed, witnessing the behaviour of what is practically a *monkey strain* of a trypanosome under peculiar conditions of maintenance, and we have recorded the appearance of certain alterations in the physiology of the strain. But caution is necessary in attempting to assign a cause for these changes, lest we ascribe to the agency of direct transmission effects due wholly, or partially, to the tissue reactions of the *Cercopithecus*. Not that there is any reason to suppose that the host factor alone, without the co-operation of direct transmission, can account for the loss of the power of a strain of trypanosomes to develop cyclically in tsetse. Provided that the passage of the monkey-strain from animal to animal is always effected by cyclically infected flies, it is a reasonable, though unproven, assumption that the parasite will retain indefinitely its power to infect the salivary glands of the fly. Presumably, too, any departure from the cyclical method will tend, sooner or later, to alter the constitution of the trypanosome ; though in the case of the Direct Transmission Strain (Duke 1921), the organism was still capable of full cyclical development in *Glossina* after eleven monkey-passages by the direct method over a period of some nine months.

On biological grounds we should expect that cyclical transmission, acting over a prolonged period, would lead to a gradual adjustment of the trypanosome to its mammalian host. An excellent example of this is the equilibrium established between ruminant game and *T. brucei*. In nature, this adjustment will be found to exist between a trypanosome and those species of animals upon which it is normally dependent. In certain *palpalis* regions there is evidence that a balance has been established between man and a member of the polymorphic group of trypanosomes. But in most, if not all, human communities where trypanosomiasis occurs, man is evidently too susceptible to qualify as an ideal host ; and while we have no evidence that game animals in their wild state are inconvenienced by their trypanosomes, in the case of man the difficulty is rather to prove the existence of any genuine tolerance.

Vertebrates in which the trypanosome provokes a rapidly fatal disease cannot be regarded as biologically perfect hosts of the parasite. Monkeys fall into this category, and so, to a great extent, does man. The strains which we have been studying in monkeys were maintained in an artificial environment. They destroyed their hosts rapidly; could not develop in tsetse; and were absolutely dependent on direct transmission for survival. The Mwanza human trypanosomes were, as a rule, rapidly fatal to their human hosts, though retaining (at the time of the first sub-inoculations into monkeys) the power to survive in tsetse; and there are reasons for believing that direct transmission played an important part in the spread of the Mwanza disease in man. To a certain extent, therefore, the monkey-strains under review are comparable with the virulent human strains in the fly-belt, *i.e.* they are both utilising vertebrate hosts to which they are imperfectly adjusted biologically. The closeness of the parallel will, of course, depend upon the extent to which vertebrates other than man are accessible to the trypanosome; in other words, to the frequency with which direct transmission from man to man is effected. In all probability, therefore, similar causes will produce similar effects, and the evolution of these virulent human strains in the fly-belt, if left to nature, will be subject to the laws that govern the behaviour of our monkey strains at the laboratory.

The *brucei-gambiense* group of trypanosomes is widely associated in nature with certain species of ruminant game. The organisms are transmitted by cyclical passage through the *Glossina* that feed upon these animals, and, apparently, no ill-effects are caused to the mammalian host. When circumstances compel a trypanosome to resort to species of mammals that possess little or no tolerance towards it, cyclical transmission by *Glossina* will tend to establish equilibrium between the parasite and its vertebrate host. If the mammal be unable to tolerate the infection and equilibrium is unattainable, the trypanosome may gradually lose its power of cyclical development, until it becomes entirely dependent on direct transmission for its propagation. For a time, however, even in highly susceptible hosts, cyclical transmission will still function, and will, no doubt, tend to delay the appearance in the strains so transmitted of the fulminant characters so favourable to the direct method.

We know of the existence in nature of three free-flagellated mammalian trypanosomes which have dispensed with cyclical transmission—*T. evansi, T. equinum* and *T. equiperdum.* As far as is known, all these organisms rely for their propagation entirely on direct transference from host to host. Surra, mal de caderas and dourine are generally recognisable by the clinical symptoms they produce. But apart from the recognition of these organisms on clinical or morphological grounds, it is a somewhat tedious process to demonstrate the inability of a trypanosome to develop cyclically in *Glossina,* and this test is not often applied to strains isolated in the field. I do not know whether posterior-nuclear forms occur in animals infected with surra ; but, in any case, the recovery from game or stock of a virulent trypanosome of the *brucei* group, incapable of cyclical development in tsetse, would inevitably suggest thoughts of *T. evansi.* It may well be that these three trypanosomes have evolved, in the manner outlined above, from fully-equipped tsetse strains of the *brucei* type.

Unfortunately for our present investigation, man is distinguished among the mammals commonly fed upon by *Glossina,* in that his relation to the trypanosomes of the *brucei-gambiense* group ranges from complete immunity to acute susceptibility. The baboon, which is exceedingly resistant to all trypanosomes, has never been found naturally infected, although it has, on rare occasions, been infected in the laboratory. This animal cannot, therefore, be made to take the place of man in experimental investigations of the polymorphic group. As far as is known, the chimpanzee is susceptible to all the trypanosomes of this group. It is man's unique position with regard to trypanosomes that makes the problems of human trypanosomiasis so difficult to solve. In *palpalis* areas, the conclusions resulting from our laboratory investigations of monkey-strains can be applied intelligibly to the study of the trypanosomes in man. Both endemic and epidemic types of the human disease are known to occur, and it is conceivable that direct transmission strains, incapable of cyclical development in tsetse, do actually exist in certain human communities. But in game-tsetse areas the case is different and more difficult to unravel. Man is very much more resistant to the trypanosomes of the *brucei-gambiense* group than are most of the species of animals upon which tsetse feed. In the case of cattle, for example, it is well known that the briefest exposure of the animals in a fly-belt results in their becoming infected ; but numbers of natives have lived for years in contact with game-tsetses without mishap. Apparently the great majority, if not all, of the cyclically-carried wild-fly strains of polymorphic trypanosomes in game-tsetse areas are innocuous to healthy man. Yet there exists, in some of these areas, a certain number of human beings who carry in their blood trypanosomes indistinguishable from the ubiquitous game organism. An attempt has been made elsewhere to discuss the etiology of these human strains (Duke, 1923b). The German view is that the human parasite, the so-called *T. rhodesiense,* is specifically different from *T. brucei.* The ability to infect man is regarded as possessing the importance of a specific character, and therefore, presumably, sufficiently deep-rooted to survive cyclical passage through tsetse. Now there is no evidence, as far as I know, to warrant this assumption. If such a genuine human parasite existed, regularly transmissible from man to man cyclically by *Glossina,* we should expect to find a considerably larger number of cases of human trypanosomiasis than actually occur in the populations of infected *morsitans* areas. We have no evidence of the existence in man in these game-tsetse areas of a mild form of trypanosomiasis analogous to that found in game animals.

There is obviously a hitch somewhere in the progress of the trypanosome as a human parasite in these regions. Its behaviour in man and the curious distribution of the disease are not adequately explained by the theory that the parasite relies mainly, if not entirely, on cyclical transmission for its passage from man to man. On the other hand, several facts point to the intervention of direct transmission. The human host succumbs to a fatal disease, in the course of which the peripheral blood is heavily charged with trypanosomes. The clinical condition is thus favourable to direct transmission, and this is further facilitated by man's gregarious habits and his hairless skin. By direct transference to another human host the trypanosome evades the ordeal of cyclical passage through the tsetse, and the attendant risk of losing its precarious hold on man.

We have seen above that a strain may undergo several passages by direct transmission without impairment of its transmissibility by the cyclical method. Possibly this observation has an important bearing on the problem before us. A series of direct passages from man to man may confer on a strain of trypanosomes, attributes sufficiently deep-rooted to survive cyclical passage through *Glossina.* In this way more or less stable cyclical human strains might arise in the wake of an epidemic.

Direct transmission offers an explanation of the sporadic distribution of the human disease in certain *morsitans* areas. In the presence of tsetse, a sick man whose blood is swarming with trypanosomes will afford excellent opportunities for the direct transference of his blood, by the fly, to his immediate neighbours ; whereas those of his trypanosomes which succeed in establishing themselves cyclically in the tsetse may, on the completion of their development in the fly, be no longer able to survive in man's blood.

There is a widespread tendency to assume that all human cases derive their infection from cyclically-infected wild flies. Great stress is laid on the fact that so-and-so became infected shortly after visiting a fly-area to hunt ; but the facts that nearly every male in the area is a hunter, and that the patient himself or his friends and relations live in more or less close contact with tsetse, are apt to be overlooked.

In such a community, parties of natives will visit the uninhabited fly-country from time to time to hunt, or to cut poles, or for some other objective, and there is nothing to prevent an early case of trypanosomiasis from participating in these excursions. In the presence of hungry tsetse such a man is a menace to his fellows. One can hardly imagine conditions more ideal for the distribution of the sick man's trypanosomes by the direct method than those obtaining during the later stages of a native hunt in *morsitans* country. I believe that a ' clean ' native entering one of the so-called infected *morsitans* areas is much more likely to be infected by the fly if one or more of his companions are already suffering from trypanosomiasis, than if he goes into the fly-country alone or with ' clean ' companions. Until we have irrefutable evidence that ' clean ' natives, from a ' clean ' area where there is no fly and no human trypanosomiasis, can contract the disease by entering a fly-area where there are no people, we should at least maintain an open mind about the relative importance of cyclical and direct transmission in the spread of human trypanosomiasis in game-tsetse areas.

In the present state of our knowledge we may suppose that, under circumstances which are not yet understood, the game trypanosome, *T. brucei*, can establish itself in man, using him as an adventitious host and causing a fatal disease, a feature of which is a heavy infestation of the peripheral blood. The parasite thus stands an excellent chance of being transmitted mechanically to fresh human hosts, and of maintaining intact those qualities, whatever they may be, which originally determined its establishment in man.

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